Cell, Vol. 92, 601–602, March 6, 1998, Copyright ©1998 by Cell Press

Letter to the Editor

Is the Putative Chloroquine Resistance Mediator CG2 the Na^+/H^+ Exchanger of *Plasmodium falciparum*?

The genesis of chloroquine-resistant *Plasmodium falciparum*, which has severely complicated the clinical management of tropical malaria, has been the focus of intense scientific investigation since it first emerged about 40 years ago. Although several models have been proposed to explain the chloroquine-resistant (CQR) phenotype (Krogstad et al., 1987; Ginsburg and Stein, 1991; Martiney et al., 1995), the mechanism, as well as the genetic determinant(s), has remained elusive. Using a genetic approach, a 400 kb region of the *P. falciparum* chromosome 7 was found to segregate with the CQR phenotype (Wellems et al., 1991). Fine scale analysis of this locus has recently identified a promising candidate gene, *cg2*, of hitherto undefined function, encoding a ~330 kDa transmembrane protein located at both the parasite plasma membrane and the food vacuole (Su et al., 1997).

We have recently shown that chloroquine is taken up, and concentrated by, the action of the *P. falciparum* sodium/hydrogen ion exchanger (PfNHE) (Sanchez et al., 1997; Wünsch et al., 1998), a plasma membrane–associated protein involved in pH and cell volume regulation. This protein imports chloroquine, during an activation phase induced by chloroquine itself, where the sodium ion gradient across the parasite's plasma membrane appears to provide the energy necessary for chloroquine accumulation. The CQR phenotype was found to be genetically linked, using the same parental and progeny clones as the Su et al. study (1997), with changes in biochemical and physiological properties of the PfNHE. CQR parasites have an NHE with both a lower affinity for chloroquine and a reduced transport rate. Moreover, their NHE is in a constitutively activated state, rendering this protein incapable of importing chloroquine since no further activation by chloroquine is possible (Wünsch et al., 1998).

Given that complex polymorphisms within *cg2* as well as changes in the biochemical and physiological properties of the PfNHE are all genetically linked with the CQR phenotype, this begs the question: Is CG2 the *P. falciparum* NHE? CG2, however, does not share obvious homologies with any known NHE. This is not surprising since physiological data suggest that the PfNHE is not a classical electroneutral NHE, as found in higher eukaryotes, but rather an electrogenic amiloride-sensitive NHE, reminiscent of those found in invertebrates. As no electrogenic NHE has thus far been cloned, a standard homology search would not have revealed CG2's identity.

A closer look at CG2, however, reveals certain structural and functional features we would expect to find in the PfNHE, including a consensus amiloride-binding site and a region homologous to a sodium/hydrogen ion transport domain of eukaryotic NHEs (Figures 1 and 2). The locations of these sites are spatially conserved and lie at appropriate regions of CG2 predicted with high probability to be transmembrane domains, of which CG2 contains 10–12 typical of NHEs (Figure 1). CG2's size and cellular location are also consistent with its role as an electrogenic NHE, which are much larger than electroneutral NHEs and tend to be located at both plasma and vacuolar membranes (Couet et al., 1993; Kimura et al., 1994), a reflection of their role as pleiotropic transporters for a wide range of mono- and divalent cations (Ahearn, 1996). That CG2's putative sodium/hydrogen ion transport domain contains additional amino acids may be attributed to its electrogenic nature. CG2 also contains the R/K-X-G-R/K-R/K motif found in many metabolite proton or sodium ion symporters as well as

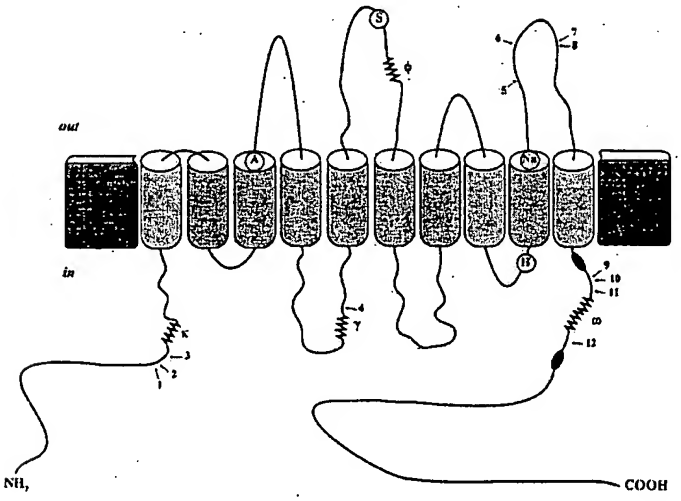

Figure 1. Predicted Topology of CG2

Regions of CG2 homologous to the sodium/hydrogen ion exchange domain (delineated by Na^+ and H^+, residues 1837–1908), a high-affinity amiloride-binding site (A, 646–652), the symporter consensus sequence (S, 1132–1136), and Ca^{2+}/calmodulin-binding sites (filled ovals, 2138–2155 and 2486–2503) are indicated. The sites of the point mutations (numbered 1 to 12), as well as length polymorphisms within repeat regions (κ, γ, φ, ω), are shown according to Su et al. (1997). High-confidence transmembrane domains (prH > 5 with Rel > 5; Burkhard et al., 1995) are shown in a dark shade, while lower confidence transmembrane domains (prH < 4 with Rel < 4) are shown in a lighter shade (residues: 570–591, 598–617, 639–659, 796–815, 1016–1036, 1291–1310, 1674–1694, 1785–1802, 1881–1901, 2116–2136).

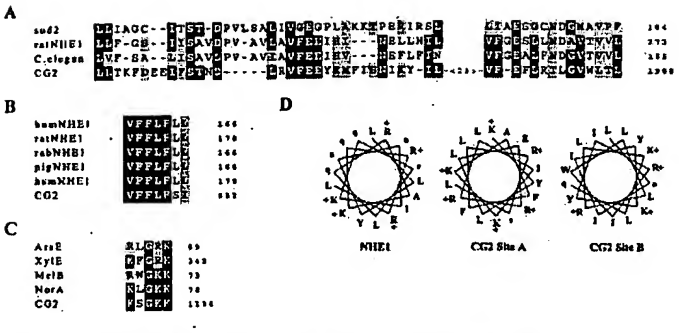

Figure 2. Alignment of Regions of Homology between CG2, NHEs, and Symporters

(A) Alignment of the sodium/hydrogen ion exchange domain of NHEs from *Schizosaccharomyces pombe* (sod2, P33036), rat (ratNHE1, P26431) and *Caenorhabditis elegans* (*C. elegan*, S30910) with CG2 (AF030693). Black boxes indicate identical amino acids, gray boxes conserved amino acid substitutions.

(B) Alignment of high-affinity amiloride-binding domains of NHEs from human (humNHE1, P19634), rat, rabbit (rabNHE1, P23791), pig (pigNHE1, P48762), and hamster (hamNHE1, S30198) with CG2.

(C) Alignment of the symport domain from the arabinose (AraE, P09830), xylose (XylE, P09098), melibiose (MelB, P30878) transporters and the quinolone efflux pump (NorA, P21191) with CG2.

(D) Helical wheel diagram representations of the Ca^{2+}/calmodulin-binding sites of human NHE1 (Yamaguchi et al., 1990) and two putative sites within CG2 (A: 2138–2155; B: 2486–2503). These sites are characterized by the distribution of clustered positively charged amino acids (±), separated by hydrophobic ones (capital letter), across a helical structure (Bertrand et al., 1994).

the bacterial tetracycline/proton exchangers (Figures 1 and 2C) (Yamaguchi et al., 1990; Yoshida et al., 1990), but which is absent in electroneutral NHEs. The presence of this motif may in part explain the unique ability of the PfNHE to transport the divalent cation chloroquine.

A constitutively activated NHE, as is associated with the CQR phenotype, can principally arise from mutations within its pH sensor or C-terminal regulatory domain, the latter being the site of action for kinases, accessory binding proteins, and Ca^{2+}/calmodulin (Wakabayashi et al., 1997). The point mutations 1 and 3 found in almost all CG2 from CQR parasites replace predicted cytoplasmically located histidines for glutamines, changing the charge profile of putative pH sensors in a manner consistent with constitutive activation of an NHE (Wakabayashi et al., 1997). Point mutations 9 to 12, and the length polymorphism of the ω repeat, all fall within a region predicted to be a C-terminal regulatory domain, as suggested by the presence of two putative Ca^{2+}/calmodulin-binding sites (Figures 1 and 2D) (Bertrand et al., 1994). A third group of point mutations, 5 to 8, map close to the putative sodium/hydrogen ion transport domain, in a region that may be involved in chloroquine binding and transport, thereby accounting for the reduced affinity of this protein for chloroquine in CQR parasites.

The probability of a specific 5–amino acid motif to occur by chance in a protein of CG2's size and amino acid composition is 8×10^{-4}. Therefore, it is unlikely that the presence of the amiloride-binding motif, in a protein we would predict to bind amiloride, is serendipitous. Moreover, the combination of all the sites identified, their correct spatial organization, as well as the location of the mutations within regions predicted to be important for NHE activity, provides strong circumstantial evidence that CG2 is the *P. falciparum* NHE. Final proof would necessitate functional studies, such as the reconstitution of CG2 in liposomes or its expression in a heterologous system.

Acknowledgment

This work was supported by grants from the BMBF and the DFG.

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